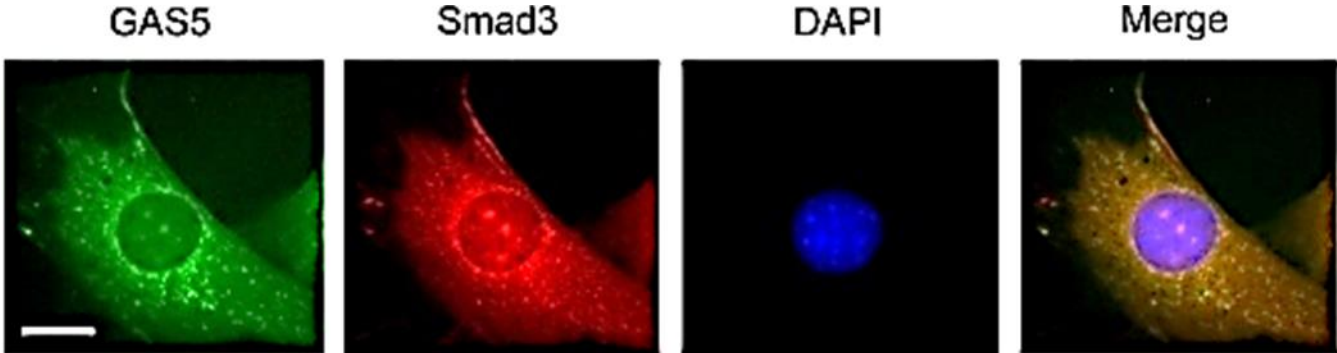# The long non-coding RNA GAS5 regulates TGF-β-induced smooth muscle cell differentiation via RNA-Smad binding element

Rui Tang, Gui Zhang, Yung-Chun Wang, Xiaohan Mei, and Shi-You Chen

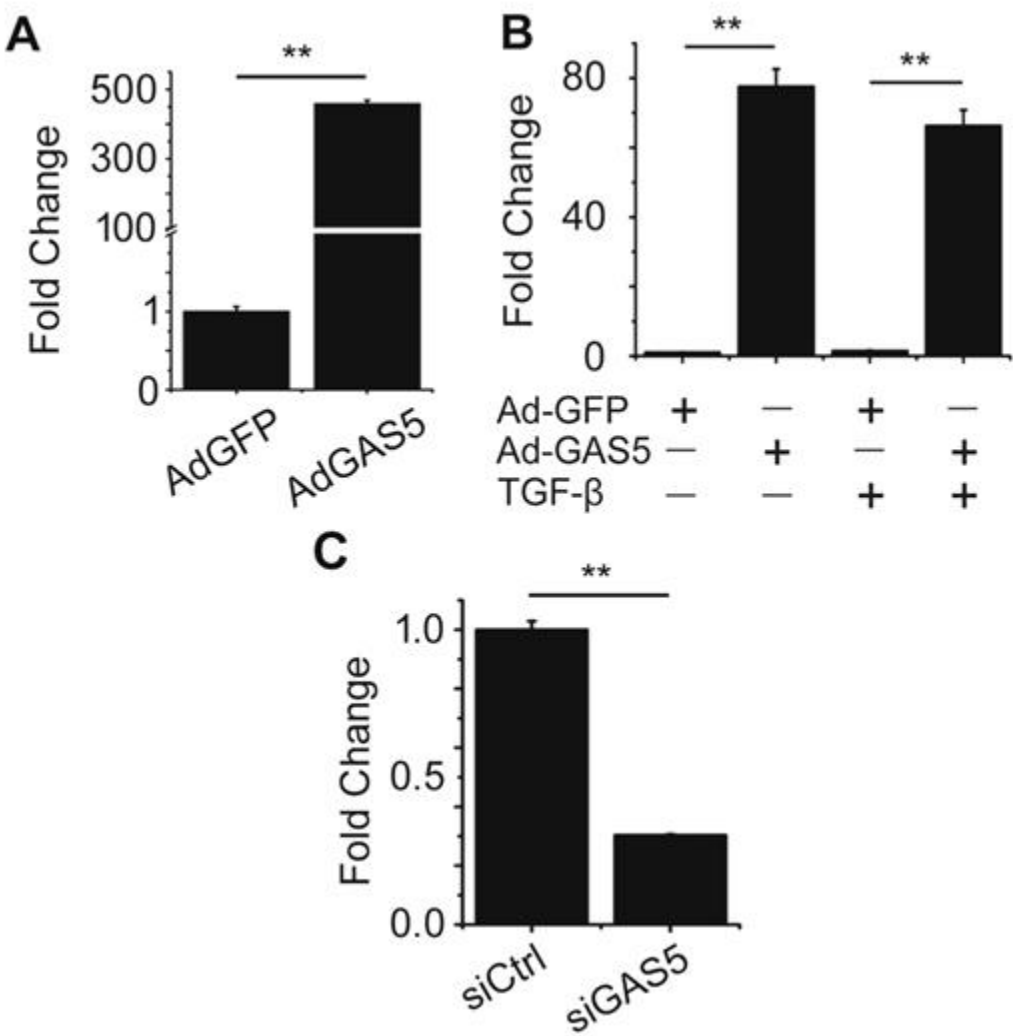Department of Physiology and Pharmacology, University of Georgia, Athens, GA

Supplemental Materials

Supplemental Figure 1

Figure S1: GAS5 (green) co-localized with Smad3 (red) in 10T1/2 cells. Shown are fluorescence in situ hybridization for GAS5 and immunostaining for Smad3. Bar: 20 μm.
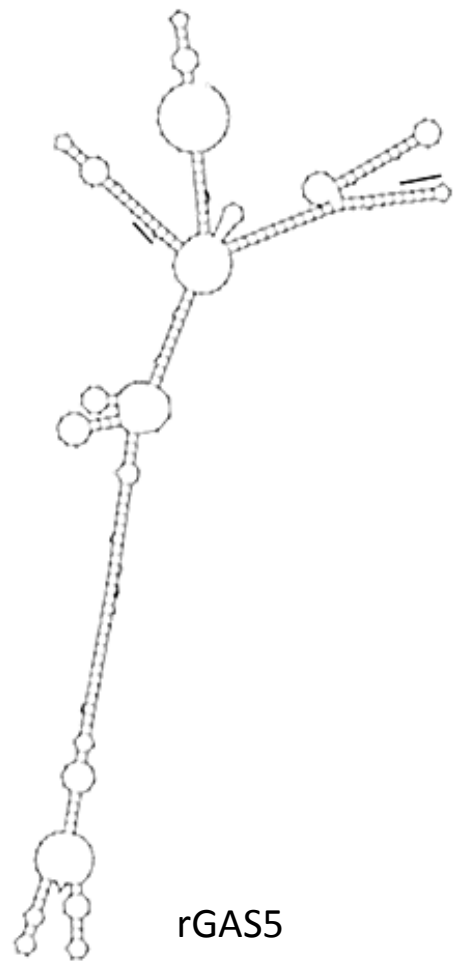
Figure S2: Overexpression and knockdown efficiency of GAS5 gene. A) GAS5 adenoviral transduction (AdGAS5) dramatically increased GAS5 expression in 10T1/2 cells. **B)** GAS5 adenoviral transduction (Ad-GAS5) increased nuclear GAS5 levels in both untreated or TGF-β-treated 10T1/2 cells. **C)** GAS5 expression was knocked down by its siRNA (siGAS5). The data present the mean±s.e.m. **P<0.01 (Student's t-test).

A

B
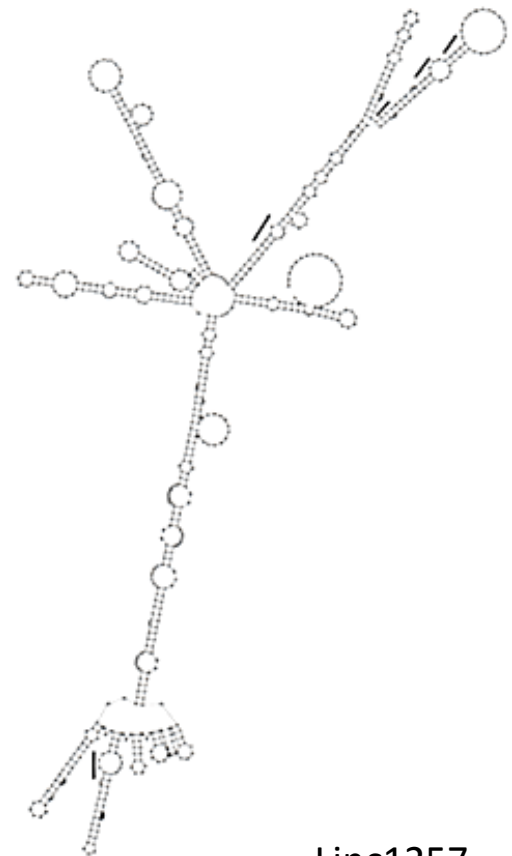
rGAS5

Linc1257

Figure S3: Predicted secondary structure of lncRNAs. A) Secondary structure of rat GAS5 (rGAS5). **B)** Secondary structure of Linc1257. Underlines indicate the locations of rSBE elements.

Supplemental Table S1: Primers used for PCR analysis, in vitro transcription, and vector construction.

mmu-U6-F	CTC GCT TCG GCA GCA CA
mmu-U6-R	AAC GCT TCA CGA ATT TGC GT
mmu-Adapt33-F	GGA GCT CTC GAT GTG ACC TG
mmu-Adapt33-R	TCT CTC ATG AGG ACA GCG GA
mmu-AK082072-F	AGA AGA TGC CCA TGA CAG CAA
mmu-AK082072-R	GGA GCA CAG AGA TCA GCA CAT
mmu-antiPeg11-F	CTC CAC TCG AGG GTA CTC CA
mmu-antiPeg11-R	TCC AAA CCG ACG ACG AAA CT
mmu-B2SINE-F	GGC TGG TGA GAT GGC TCA GT
mmu-B2SINE-R	AAA GAT TTA TTT ATT TAT TAT ATG
mmu-BACE1AS-F	ATG CCC CAT CCT CAT CCC AA
mmu-BACE1AS-R	TGC TTG GCA TAC CCA AAT GC
mmu-BC1-F	AAG GAG TTG GGG ATT TAG CTC
mmu-BC1-R	AAT AAT GAT GTA ATT TGA TTT AC
mmu-BORG-F	AAT CCC AGC AGC CGT GTA AA
mmu-BORG-R	GTC TTT GCT CAC TTC CCC CA
mmu-CDR1AS-F	CAT GTG TCT TCC TTC ACC TCC A
mmu-CDR1AS-R	CGG CAG ACA CCA GAA AAC CT
mmu-Dio3os-F	ATG GGA CAC TTG GGA AAC CC
mmu-Dio3os-R	AGG TGT GGG GTG TAG CCT AT
mmu-GAS5-F	GGA GGT TGG TTC TGC GTG TA
mmu-GAS5-R	CGC ATG CTG AGT CGT CTT TG
mmu-H19-F	GCA GGT AGA GCG AGT AGC TG
mmu-H19-R	AGA CCT GGC CTA GTC TCC AG
mmu-HOTAIR-F	GGC TGC CTG AGT TCT TTT GC
mmu-HOTAIR-R	TGC GGT GGA GAT AGA TGT GC
mmu-HOTTIP-F	TCC CGC TTT GTA CAG GGA AC
mmu-HOTTIP-R	GAG GGG CTT GCT ACA CCT TT
mmu-Jpx-F	CTC AGG TGG TTT TTG CGC TG
mmu-Jpx-R	CCT GCC AGT CAC AAG GCT AA
mmu-lincMD1-F	CTC TGG CTG GTC AAA CGG AA
mmu-lincMD1-R	CCC TGC TGT AGC TGG TTG AA
mmu-linc1257-F	AAC TGG GTG ATA CGC ACT GG
mmu-linc1257-R	CCA AAG CTC GGT CAT CCC TT
mmu-linc1368-F	TTG GCA GAG CTC AGT GGA TTT
mmu-linc1368-R	TAA AGC AGC CCA AGA TGC AGA
mmu-linc1582-F	GTG TTT CAC TGT GCA TCG CC
mmu-linc1582-R	TGC ATG CGG CTG TGG ATA AA
mmu-linc1609-F	AGC GCC CTC ATT TAA CAC CA
mmu-linc1609-R	TGT GTG GGC ACA AAG GTC AT
mmu-lincRNA-Cox2-F	AGC TGC ACG CAC ATT AGA GA
mmu-lincRNA-Cox2-R	GCA GAA GCA GAG AGG TGA GG

mmu-lincRNA-p21-F	GAC CAG AAC TGG AGC CAA CA
mmu-lincRNA-p21-R	GCC AAC TGG AAG CAA CTG TG
mmu-LXRBSV-F	CTG CCT ACA TCG TGG GTG AG
mmu-LXRBSV-R	AAG CGG ACC AAA AGA CAG GG
mmu-MEG9-F	ACA AAA GGG ATG ATG CCG GT
mmu-MEG9-R	CAT CCA GGA TTG GTC CCC AC
mmu-megamind-F	TGA AAG GCC CCA TTC AGA CC
mmu-megamind-R	TCG CTG ATA GAC GGA TGC AC
mmu-Mistral-F	GGC CAA TAC CTG CAA ACC AT
mmu-Mistral-R	ACC GAG AAG CAG GTG AGC TA
mmu-Mrhl-F	TGG TGT GAC CCA CAA CAT GA
mmu-Mrhl-R	GGA AGG GCC AGC TTC CTA AA
mmu-MSUR1-F	GTG GAG CGA TTT GTC TGG TT
mmu-MSUR1-R	ACG CTG AGC CAG TCA GTG TA
mmu-Msx1as-F	TAA AGA GAG TCT TGC TGG GGT G
mmu-Msx1as-R	TTC TCA AGT GCC TCA CAG TGC
mmu-NEAT1-F	TTT GCC TAG GTT CCG TGC TT
mmu-NEAT1-R	TAA CCT CAG AGT GAG GGG CA
mmu-Nespas-F	TGG GGG CCA AGC TTT TTG AT
mmu-Nespas-R	ATC ATG AGC CCA AGG GAT GG
mmu-NRON-F	CTT CTG TCC AAG CCA CCC AT
mmu-NRON-R	ATG GCC CAT GAG GGA AAC TC
mmu-Otx2os1-F	GCA ACT CTG TCC GCT TGT TG
mmu-Otx2os1-R	CCC TAG ACG TCT GCA AAG CA
mmu-PINC-F	CTG CAG GAA GGA TAC CGT GG
mmu-PINC-R	GAT GGA TCA TCC GGG CAT GT
mmu-Pldi-F	GGG ATA GGA AAG ACG CGA GG
mmu-Pldi-R	TGA GTT GGC CCT GGA ATG AC
mmu-RepA-F	CGA GGG TAC ACT TGG TGC AT
mmu-RepA-R	TGG CAA CCA ACA CTT CCA CT
mmu-Rmst-F	GGC TAC GTG TGG GTA CAG TT
mmu-Rmst-R	TGC TGC TCC TGT CCT TTA GC
mmu-RNCR3-F	CTG CAG AAA TAA GCC ACC GC
mmu-RNCR3-R	CAG TCT GGC TTG TCC GGT AG
mmu-Six3os-F	GTC GCC TGG TTC TCT GAT GA
mmu-Six3os-R	CCT CAT CCC ACT ACG GAA CG
mmu-SRA-F	TGA TAT CAG CCG ACG CTT GG
mmu-SRA-R	GTC ATC TGC TGC ATC CCA CT
mmu-Sry-F	TTA GTG TTC AGC CCT ACA GCC
mmu-Sry-R	ATT CAT GGG GCG CTT GAC A
mmu-Tip3-F	TGG CCG AGC AGA TGT GAA AG
mmu-Tip3-R	AGC TCA CAG CCA GCT ACA AA
mmu-VL30-F	TAG ACG TGT CCA GGT GTC CA
mmu-VL30-R	GAT CTC GCA ACA AAA CAC GA

mmu-YRNA-F	GCT GGT CCG AAG GTA GTG AG
mmu-YRNA-R	CTA GTC AAG TGC AGT AGT GAG AAG G
mmu-Zfas1-F	GGC TCG GGG ACT ACA TTT CC
mmu-Zfas1-R	CCG GAA CGA AAG GAC GAG AG
mmu-SM22-F	GGT CCA TCC TAC GGC ATG AG
mmu-SM22-R	CCT ACA TCA GGG CCA CAC TG
mmu-SMA-F	AAT GGC TCT GGG CTC TGT AAG
mmu-SMA-R	CAC GAT GGA TGG GAA AAC AGC
mmu-Col1a-F	TCC TTC TGG TCC TCG TGG TCT C
mmu-Col1a-R	AGC CTC GGT GTC CCT TCA TTC C
mmu-CNN1-F	AAT ACG ACC ATC AGC GGG AG
mmu-CNN1-R	ATG AAG TTG TTC CCG ATG CG
mmu-GAS5-F1-pshuttle-F	ACG CCC GGG CCT CGA GAC CAT GCT TTC GGA GCT GTG CGG CAT TCT GAG C
mmu- GAS5-F1-pshuttle-R	CCT TGT AGT CCT CGA GAA GAA AAA TGT TAA TCT TTA GTA GTT CTA CTT CC
mmu-GAS5-F2-pshuttle-F	ACG CCC GGG CCT CGA GAC CAT GAG GAA TGG CAG TGT GGA CC
mmu- GAS5-F2-pshuttle-R	CCT TGT AGT CCT CGA GTG TTC TAG TCA TGC CCC TC
mmu-GAS5-F3-pshuttle-F	ACG CCC GGG CCT CGA GAC CAT GTA ATG ACC ACA AAG CCC ATG ATG G
mmu- GAS5-F3-pshuttle-R	CCT TGT AGT CCT CGA GGG GGG GGG GAA TCT TTA TGT AAA AAC TGC
mmu-GAS5-F4-pshuttle-F	ACG CCC GGG CCT CGA GAC CAT GCA GGA CTC GTC AGG AAG CTG
mmu- GAS5-F4-pshuttle-R	CCT TGT AGT CCT CGA GTA CCT TTC AAA CTG TCT ATC C
mmu-GAS5-F5-pshuttle-F	ACG CCC GGG CCT CGA GAC CAT GAG TAT TGA AAA ACA CTT GAA TTT GGG
mmu- GAS5-F5-pshuttle-R	CCT TGT AGT CCT CGA GTT AAT ACC TGT AAG AAA TTA CAC TCC
mmu-GAS5-F6-pshuttle-F	ACG CCC GGG CCT CGA GAC CAT GTG GGT CAC CTC AAG TGA AGG
mmu- GAS5-F6-pshuttle-R	CCT TGT AGT CCT CGA GCA TGT TAT AAT ACA CTT TAA TGG
mmu-GAS5-pshuttle-F	ACG CCC GGG CCT CGA GAC CAT GCT TTC GGA GCT GTG CGG CAT TCT GAG C
mmu-GAS5-pshuttle-R	CCT TGT AGT CCT CGA GCA TGT TAT AAT ACA CTT TAA TGG
mmu-GAS5fl-(T7)-F	GAG TAA TAC GAC TCA CTA TAG GGC TTT CGG AGC TGT GCG GCA TTC
mmu-GAS5fl-(T3)-R	GAG ATT AAC CCT CAC TAA AGG GAC ATG TTA TAA TAC ACT TTA ATG G
mmu-G5-FISHprobe-T7	GAG TAA TAC GAC TCA CTA TAG GGA ACA GTG CCT ATG TGG ACG G
mmu-G5-FISHprobe-T3	GAG ATT AAC CCT CAC TAA AGG GAC TAT CGT CAC CCC AGC TGT C
SBERNA-CAGAC-T7-F	GAA ATT AAT ACG ACT CAC TAT AGG GCA GAC AGC AGA CTC AAG AGG TCT GGA GTC TGA ACA GAC AGC AGA CTC AAG AGG TCT GGA GTC TG
SBERNA-CAGAC-T7-R	CAG ACT CCA GAC CTC TTG AGT CTG CTG TCT GTT CAG ACT CCA GAC CTC TTG AGT CTG CTG TCT GCC CTA TAG TGA GTC GTA TTA ATT TC
SBERNA-CAGGG-T7-F	GAA ATT AAT ACG ACT CAC TAT AGG GCA GGG AGC AGG GTC AAG AGC CTG GAC CCC TGA ACA GGG AGC AGG GTC AAG AGC CTG GAC CCC TG

SBERNA-CAGGG-T7-R	CAG GGT CCA GGG CTC TTG ACC CTG CTC CCT GTT CAG GGT CCA GGG CTC TTG ACC CTG CTC CCT GCC CTA TAG TGA GTC GTA TTA ATT TC
SBERNA-CAGAG-SBE-F	GAA ATT AAT ACG ACT CAC TAT AGG GCA GAG AGC AGA GAG CAG AGA GCA GAG TCA GAG CTC TGA CTC TGG ACT CTG
SBERNA-CAGAG-SBE-R	CAG AGT CCA GAG TCC AGA GTC CAG AGC TCT TGA CTC TGC TCT CTG CTC TCT GCT CTC TGC CCT ATA GTG AGT CGT ATT AAT TTC
SBERNA-CAGGC-SBE-F	GAA ATT AAT ACG ACT CAC TAT AGG GCA GGC AGC AGG CTC AAG AGG CCT GGA GCC TGA ACA GGC AGC AGG CTC AAG AGG CCT GGA GCC TG
SBERNA-CAGGC-SBE-R	CAG GCT CCA GGC CTC TTG AGC CTG CTG CCT GTT CAG GCT CCA GGC CTC TTG AGC CTG CTG CCT GCC CTA TAG TGA GTC GTA TTA ATT TC
CAGAC-SBERNA-RT	CTC AAC TGG TGT CGT GGA GTC GGC AAT TCA GTT GAG CAG ACT CCA GAC
CAGAC-SBERNA-R	TGG TGT CGT GGA GTC G
CAGAC-SBERNA-F	ACA CTC CAG CTG GGG AAA TTA ATA CGA CTC ACT ATA GGG
mmu-SM22-ChIP-SBE-F	GGT GTT GAG CCA AGC AGA C
mmu-SM22-ChIP-SBE-R	CGA GTT GCA TTA GCC CTG G
mmu-linc1257- pshuttle-F	ACG CCC GGG CCT CGA GAC CAT GAA CTT TAA GCA CAC GTG AGC GTC C
mmu-linc1257- pshuttle-F	CCT TGT AGT CCT CGA GCC AGG ACA TGT CCC AAA GCT CGG
GAS5-caggg-F1-F	GAA ATT AAT ACG ACT CAC TAT AGG GGAGCTTAGTTCAGGGCACAAGCTGGAAACTTTAGGAGGTTGGTTCT CAAGAGGTGACTGCACCTTTGTTTCTGAGGTGCCTGGATGGAGGCTC
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GAS5-caggg-F1-MUT-F	GAA ATT AAT ACG ACT CAC TAT AGG GGAGCTTAGTTgtcct CACAAGCTGGAAACTTTAGGAGGTTGGTTCTCAAGAGGTGACTGCAC CTTTGTTTCTGAGGTGCCTGGATGGAGGCTC
GAS5-caggg-F1-MUT-R	GAG CCT CCA TCC AGG CAC CTC AGA AAC AAA GGT GCA GTC ACC TCT TGA GAA CCA ACC TCC TAA AGT TTC AGC TTG TGG agg acA ACT AAG CTC CCT AT AGT GAG TCG TAT TAA TTT C